MICROWAVE ASSISTED ENZYMATIC TRANSESTERIFICATION OF WASTE COOKING OIL & RUBBER SEED OIL

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ABSTRACT

This thesis presents biodiesel production from rubber seed oil via enzymatic process using Immobilized *Lipase Candida Rugosa*. The objectives of this study is to produce biodiesel fuels from waste cooking oil and rubber seed oil that comply with ASTM standard under optimum conditions of transesterification process such methanol to oil ratio, reaction temperature, mixing speed and microwave power using commercial and purpose made Immobilized *Lipase Candida Rugoas* enzyme in microwave reactor. This research also focus on production of biodiesel economically from non-edible renewable resources which using rubber seed oil and waste cooking oil as raw material instead of edible oil that competed with food sources. From the screening of three type of reactors, which were batch, microwave and ultrasonic, microwave reactor produced highest methyl ester in enzymatic transesterification. Microwave irradiation was applied in immobilization process. of *Lipase Candida Rugosa* enzyme was immobilized onto silica gel by cross-linking immobilization. The enyzme loading of using cross-linking method in this study was 47 % which is lower than reported by other researchers but the enzyme activity of purpose made immobilized lipase enzyme was 33 % higher than commercial immobilized lipase enzyme. From the parameter study of enzymatic transesterification process of waste cooking oil in microwave reactor, the optimum conditions were 6:1 methanol to oil ratio, 40°C reaction temperature, 150 rpm of mixing speed and 100 W microwave power. Enzyme leaching and enzyme recyclability has also been studied. Microwave irradiation increased the methyl ester production but, 25 % of this work immobilized enzyme was leached from silica support and reduced 21 % of its activity after 8 cycles. Compared with commercial immobilized lipase enzyme, purpose made immobilzied lipase enzyme showed higher stability. The purpose made immobilized enzyme than was been tested in transestrification process of palm oil, rubber seed oil and waste cooking oil. The resuls shows that the palm oil produced highest methyl ester, followed by rubber seed oil and waste cooking oil. 99 % of methyl ester was successfully produced using crude rubber seed oil, 1 wt. % purpose made immobilized lipase enzyme with 6:1 methanol to oil ratio, 40°C reaction temperature, 150 rpm of mixing and 100W of microwave power irradiation. The results also complied with ASTM standard and produced significant data.

ABSTRAK

Tesis ini berkenaan dengan pengeluaran biodiesel daripada minyak biji getah secara enzimatik menggunakan enzim pegun *Lipase Candida Rugosa*. Objektif tesis ini adalah menghasilkan minyak biodiesel daripada minyak buah getah dan minyak masak terpakai yang mematuhi piawaian ASTM dalam keadaan tindak balas yang optimum dalam penghasilan biodiesel seperti kadar nisbah methanol kepada minyak, suhu kadar tindak balas, kelajuan pengacau, dan juga tenaga mikrogelombang dengan menggunakan enzim pegun komersial dan juga enzim pegun yang dihasilkan sendiri di dalam reaktor mikrogelombang. Melalui proses penyaringan tiga reaktor yang berbeza iaitu reaktor kelompok, reaktor ultrasonik dan reaktor mikrogelombang, reaktor mikrogelombang menunjukan pretasi yang paling bagus dalam penghasilan metil ester perbanding yang lain. Reaktor mikrogelombang juga digunakan dalam penghasilan ezim pegun secara perangkaian silang. Kaedah ini menghasilkan kadar serapan sebanyak 47% enzim ke dalam gel silika. Walaupun kadar serapan ini adalah lebih rendah berbanding yang pernah direkodkan oleh pengkaji yang lain, kadar aktiviti enzim pegun ini lebih tinggi daripada enzim pegun komersial. Walau bagaimanapun kaedah ini menghasilan enzim pegun yang lebih baik daripada enzim pegun komersial yang digunakan dalam tesis ini. Ujian pengoptimuman paremeter dibuat menggunakan reaktor mikrogelombang dan parameter yang paling optimum adalah kadar nisbah metanol kepada minyak 6:1, suhu tindak balas 40°C, 150 rpm kelajuan pengacau dan 100 W tenaga mikrogelombang.. Enzim ini juga memiliki kadar melarut resap sebanyak 25 % dan ini menyebakan kadar aktivity enzim pegun menurun sebanyak 21 % selepas 8 kali digunakan. Enzim pegun yang telah berjaya dihasilkan itu kemudiannya diuji dalam proses penghasilan biodiesel menggunakan minyak buah getah, minyak masak terpakai dan minyak kelapa sawit mentah dalam keadaan kadar tidak balas yang paling optimum. Minyak kelapa sawit mentah menghasilkan metil ester yang paling tinggi diikuti oleh minyak biji getah dan minyak masak terpakai. Sebanyak 99 % metil ester telah terhasil daripada minyak buah getah dengan menggunakan 1 % berat enzim pegun yang dihasilkan daripada kajian ini dalam keadaan kadar tindak balas yang optimum iaitu kadar nisbah methanol kepda minyak 6:1, suhu tindak balas 40°C, 150 rpm kadar kelajuan pengacau dan 100 W tenaga reaktor mikrogelombang. Biodiesel yang terhasil jugak mematuhi piawaian ASTM dan memilki data yang signifikan.

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LIST OF ABBREVIATIONS

ASTM	America Standard for Testing & Materials
CO_2	Carbon Dioxide Gas
FAME	Fatty Acid Methyl Ester
CH_3ONa	Sodium Methoxide
NaOH	Sodium Hydroxide
KOH	Potassium Hydroxide
FFA	Free Fatty Acid
H_2SO_4	Sulphuric Acid
APTES	3-aminopropyltriethoxysilane
GC	Gas Chromatography
BSA	Brovine Serum Albumin
MOPS	3-(N-morpholino) propane sulfonic acid
FTIR	Fourier Transform Infrared Spectrophotometer
TGA	Thermal Gravimetric Analyzer
SEM	Scanning Electron Microscope
ANOVA	Analysis of Variance

CHAPTER 1

INTRODUCTION

1.1 MOTIVATION AND STATEMENT OF PROBLEM

Biodiesel is a mixture of various fatty acid methyl ester oils produced from vegetable oil or animal fat for use as fuel in diesel engine and in compliance with American Society for Testing and Materials (ASTM) standards. Biodiesel was first introduced by Rudolph Diesel (Akoh et al., 2007) in 1900. Biodiesel is considered as the best candidate for to substitute petrodiesel after the world oil crisis in 1970's. Biodiesel can be used alone or mixed with petroleum-based diesel as an alternative efficient fuel and can be used in any diesel engine without modification (Demirbas, 2009a). The most common biodiesel blends are B2 (2% biodiesel and 98% petroleum diesel), B5 (5% biodiesel and 95% petroleum diesel), and B20 (20% biodiesel and 80% petroleum diesel) (Balat & Balat, 2010). Besides, the world oil crisis which urged the researcher to find the alternative for petroleum based fuel biodiesel also has several advantages such as portability, ready availability, renewability, higher combustion efficiency, lower sulfur and aromatic content, higher cetane number and higher biodegradability which contributed to greener fuel. The higher the blending ratio of biodiesel means the fewer the carbon emission to the environment (Fukuda et al, 2001). But biodiesel also has several disadvantages such as high viscosity of vegetable oils as compared to petroleum-based diesel (Demirbas, 2007). Consequently, it causes poor fuel atomization which yielded incomplete combustion and engine fouling (Chauha et al., 2010).

Despite of the Europian Union and United States enforcement on biodiesel use on vehicles due to it green advantages, Malaysia's biodiesel industry continued to show lack of progress until the fifth Fuel Policy was announced under the Malaysia Plan (2001–2005). This policy was revised from the earlier fourth Fuel Diversification Policy in 1981 that aims at preventing overdependence on oil as the main energy resource. The new policy among other stated to supplement the conventional supply of energy and new sources such as renewable energy will be encouraged. In this regard, the fuel diversification policy which comprises oil, gas, hydro and coal will be extended to include renewable energy as the fifth fuel, particularly biomass, and mini-hydro. Of these, biogas, municipal waste, solar biomass resources such as oil palm and wood waste as well as rice husks, on a wider basis mainly for will be used potential sources of energy electricity generation. Other will include palm diesel and hydrogen fuel (eib.ptm.org.my). Thereafter, to encourage utilization of renewable resources greater efforts had being undertaken and this had developed renewed interest in biodiesel.

Although interest in biodiesel is rapidly increasing, the process by which it is synthesized has not substantially changed in recent years. The primary commercial process used today for biodiesel production is chemical catalyzed transesterification. Catalytic reactions can use alkali catalyst, acid catalyst or enzymatic transesterification (Chen et al., 2012). Enzymatic method can be used to produce better quality biodiesel with little or no by product. Lipases are one of the biotechnologically most relevant enzymes for biodiesel production. Lipases are group of enzyme that catalyzes hydrolysis, esterification and transesterification reactions. They act on several ester compounds and their natural substrates are acylglycerols (Sayari et al., 2005). The reasons for these enzymes great biotechnological potential, besides the different types of reaction that they can catalyze are their high stability in the presence of organic solvents, the lack of need for cofactors and their ability to catalyze reactions with chemo-, region- and enantioselectivity (Akoh et al., 2007). The conventional enzymatic transesterification is not efficient and time consuming. This work aims to overcome these issues by employing a microwave irradiationand ultrasonication to improve enzymatic transesterification process. Recently, the European Union has voted against a biofuel production using first generation biofuel sources (edible

oils) such as palm oil, corn, soy bean and maize, which are also consumed as food. This had open a new avenue of producing a biodiesel using a non-food source crop such as forest seeds (i.e. *Callophyluminnophylum L.*, *Elateriospermumtapos*, *HeveaBrasiliensis*) which was used in this work.

The industry has favoured chemical catalyst because of the high convention obtained in a short time and the low cost of the catalysts. However, there are some drawbacks in the process that have encouraged researchers to look into different biodiesel production methods. The use of enzymes (lipases) as catalysts in biodiesel production overcomes the problems inherent to alkali catalysts. However, biodiesel plants using lipases are not yet industrialized because there are some challenges that are yet to be overcome before biocatalysts can be made feasible for biodiesel production such as their higher cost, biodiesel productivity and inhibition by reactants and products (Al-Zuhair et al., 2011).

Even though the commercialization of biodiesel production from biocatalyst is uncertain, the world demands for biodiesel keep increasing year by year and this attracted researcher to search for the new feedstock with optimum parameter to obtain high yield, high quality and comparable biodiesel fuel with petroleum-based diesel. This study was optimizing the transesterification process of biodiesel production from waste cooking oil and rubber seeds oil using microwave irradiation. Besides the new feedstock and optimum reaction parameter, enzyme modification is also one of the interests in this study. Lipases enzyme was chemically modified by cross linking method before immobilize into silica support.

1.2 OBJECTIVES

The goal of this research is to produce biodiesel from waste cooking oil and rubber seed oil using immobilized *Lipase Candida Rugosa* enyzme and microwave reactor. In order to archieved this goal, this reasearch aims to succed at the following objectives:

1. To Immobilize the *Lipase Candida Rugosa* enzyme into silica gel support using cross-linking technique.
2. To study the effect of parameters such as reaction time, methanol to oil ratio, reaction temperature, mixing, microwave power effect and Immobilized enzyme recyclability in microwave assisted enzymatic transesterification.
3. To produce biodiesel from rubber seed oil using immobilized *Lipase Candida Rugosa* under microwave assisted enzymatic transesterification process.

1.3 RESEARCH SCOPES

To achieve the objectives of this research, several scopes have been identified
:

1. Reactor screening between batch, ultrasonic and microwave reactor in order to choose the reactor that produce the highest methyl ester in enzymatic biodiesel production. The experiment conditions such reaction time, reaction temperature, methanol to oil ratio and mixing speed was set as constant in all reactors except the microwave power and ultrasonic power. All the parameters were taken from the literature review.
2. Rubber seed oil extraction in microwave extractor using the optimum parameters from literature review. The extracted RSO use as one of the feedstock in transesterification process.
3. Immobilization of *Lipase Candida Rugosa* enzyme into silica gel support using cross linking techniques to increase the activity of the enzyme. Enzyme loading and enzyme activity was measured using specific methods while the immobilized enzyme was characterized using Scanning Electron Microscope, Thermal Gravimetric Analyzer, Fourier Transform Infrared Spectrophotometer and Wet Laser Diffraction.
4. Microwave assisted enzymatic transesterification of waste cooking oil and extracted rubber seed oil using purpose made immobilized enzyme and commercial immobilized enzyme. The parameters are study using OFAT (one factor at one

time) method which are reaction time, reaction temperature, methanol to oil ratio, mixing speed, microwave power, enzyme leaching and enzyme recyclability.

5. The methyl ester produce then analyze using gas chromatography for methyl ester content and characterize using American Society for Testing and Materials Standard (ASTM).

1.4 SIGNIFICANT OF RESEARCH

The combination of enzymatic transesterification and microwave irradiation can produce higher methyl ester in shorter time compare to conventional enzymatic transesterification process that ususally need >70 hours to produce 80% of methyl ester. Currently, the microwave assisted enzymatic transesterification was studied statistically only by Noguire et al (2010) that use Macauba oil with immobilized novozyme 435 and Perez et al., (2014) that use Babasu oil with immobilized *Lipase Burkholderia Cepacia* enzyme. None had studied using waste cooking oil with immobilized *Lipase Candida Rugosa* enzyme in. This study utilized the use of green catalyst that discharge less waste water compared to chemical catalyst since the purification method was easier due to the easy glycerol separation. This reserach also use waste to wealth concept by using the waste cooking oil and rubber seed oil to produce biodiesel. Instead of dumping into the sewage the waste cooking oil was collected and use as feedstock while the rubber seed oil had no other application since it was non-edible. Cross linking immobilization technique was great to increase the enyme activity and stability compared to other techniques.

1.5 ORGANIZATION OF THESIS CHAPTERS

The structures of the reminder thesis are given as follows:

Chapter 2 gives a review on enzymatic biodiesel production. Detail discussion on the past researches and current researches in biodiesel production, current problems in biodiesel production and potential solution that become the objectives of study and comply with the reserach scopes. Chapter 3 gave brief explanation on materials and methods used in the study. Chapter 3 was divided into overview, materials, methodology, statistical analysis and summary. All the chemicals and enzyme used in the study was listed in materials section while methodology divided into seven subchapters which explained all the methods used in the whole experiment including analysis. Chapter 4 focuses on result and discussion of the whole study finding. Chapter 4 was divided into 8 subchapters. Subchapter 4.1 was overview of chapter 4 which a brief introduction on chapter 4. Subchapter 4.2 was discussed on comparative study of immobilized and free lipase enzyme and three different reactors which are batch, ultrasonic and microwave reactors in biodiesel production. *Lipase from Candida rugosa* enzyme microwave assisted Immobilization into silica gel by cross linking technique finding was discussed in subchapter 4.3. Subchapter 4.4 was discussed on microwave assisted transesterification of waste cooking oil using immobilized lipase enzyme study. This sub chapter focused on finding the optimum parameters in biodiesel production using immobilized lipase enzyme. Subchapter 4.5 discussed the microwave irradiation influence on immobilized lipase enzyme in biodiesel production and subchapter 4.6 was comparing the biodiesel produced from waste cooking oil and rubber seeds oil using commercial using purposed made immobilized lipase enzyme under optimum MAET reaction conditions. ASTM analysis od biodiesel produced was discussed in sub chapter 4.7 and the last sub chapter which is 4.8 was a summary of chapter 4.

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW

This chapter presents the literature of biodiesel production including the world biodiesel trend and Malaysia biodiesel trend from year 1980 to 2012. Chapter 2.3 dicussed the biodiesel transesterification and divided into 5 subchapters according to the factors that affect the biodiesel transesterification which are catalyts, feedstock, reactor, solvent and reaction conditions. Subchapter 2.3.1 discussed the type of catalyst used in biodiesel production which are chemical catalyst, biocatalyst and including the biocatalyt immobilization techniques. Subchapter 2.3.2 discussed the feedstock used in biodiesel production which can be choose between edible and non-edible oil. While subchapter 2.3.3 disccused the reactor used in biodiesel production such microwave reactor, ultrasonic reactor and batch reactor. The solvent used in biodiesel transesterification was disccused in subchapter 2.3.4 and the last subchapter which is subchapter 2.3.5 disccused briefly the reaction conditions used in biodiesel production. All the finding from the literaure review then being summarized in the last chapter which is chapter 2.4.

2.2 INTRODUCTION

World climate changes are worsening day by day. There were a lot of pollutions worldwide that going out of the control despite the efforts from such as European countries to reduce the CO_2emissions using green technologies and policies. From the U. S. Energy Information Administration statistic, the world CO_2 emission increased 43.42% from 1980 to 2011. This resulted from the high energy consumption worldwide. The major CO_2 emission came from the transportation sector that used petroleum & diesel fuels. However the world petroleum depletion was an ignition source to biodiesel mass researches and productions. According to the BP Annual Statistic Review of World Energy, it was estimated that the world petroleum reserves was enough to produce petroleum in another 42 years (Balat & Balat, 2010).

Biodiesel was introduced to the world a century ago by Rudolf Diesel in year 1900 at Paris World Exhibition (Sahoo & Das, 2009).Rudolf demonstrated the used of peanut oil directly to the diesel engine(Panwar et al., 2010). At that time, lack of biodiesel technology and information make it less interesting compared to this day. Direct use of vegetable oil in diesel engine cause several problems to the engine such as poor atomization of fuels, incomplete combustion and engine coking (Karmakar et al., 2010) thus, transesterification of vegetable oil was one of the methods to reduce the viscosity of the oil and improved the quality of biodiesel and make its more suitable to use in diesel engine.

Biodiesel has several advantages over petroleum based diesel fuels such renewable, high combustion than vegetable oil, less toxic by producing low sulfur and aromatic content, biodegradable and high flash point (Balat & Balat, 2010) (Ramezani et al., 2010). However, from the ASTM analysis shows that the quality of biodiesel is still low compared to petroleum based diesel fuels thus, the biodiesel was blend with diesel to produce high qualityof biodiesel that can increase engine performance (Balat & Balat, 2010). Currently the marketed blend biodiesel was B5 and B20. B5 means 5% of biodiesel was blended with another 95% of diesel from petroleum based while B20 consisted of 20% of biodiesel and another 80% of petroleum based diesel.

2.2.1World Biodiesel Trend

World petroleum demand greatly increase over the past decades due to the increase in population and vehicles. World population recorded in year 2011 is 6.94 billion and in year 1980 it is just 4.4 billion. The population increase lead to the increase of the human basic needs such foods and energy. From the U. S. Energy Information Administration statistic data that show in figure 2.1, the petroleum demand increased year by year and the recorded from year 1980 to 2012 shows that the petroleum demand reached 89million barrels per day at 2012 despite the petroleum reserve depletion.

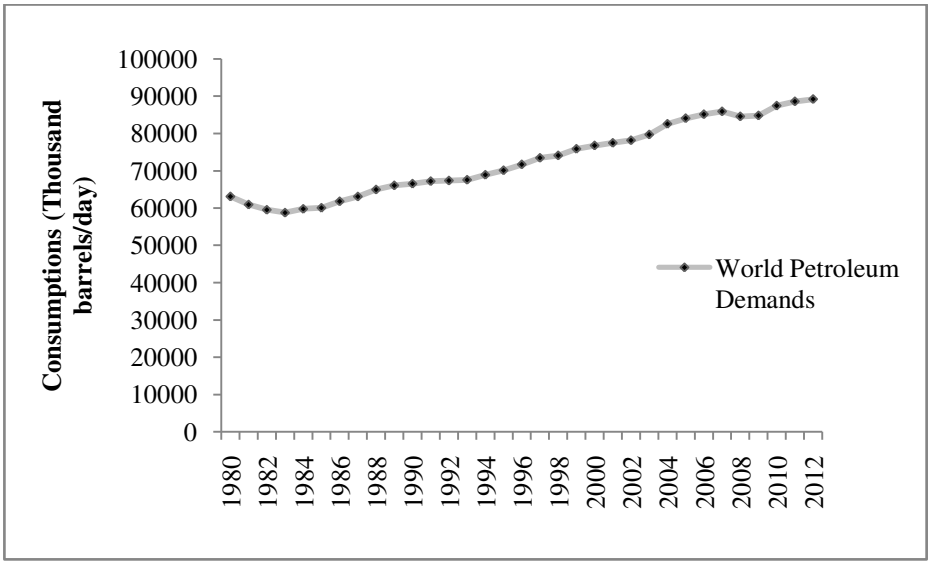

Figure 2.1: World Petroleum Demands by EIA statistic graph. (http://www.eia.gov)

These make the research, production and policies on biodiesel imperative. However the world biodiesel production and demands are still far behind the petroleum demand. Figure 2.2 shows that in year 2011, only 1.9 million barrels per day of biodiesel had been produced compared to 89 million barrels per day of petroleum production, it was 97% of margin. The exponential graph also clearly shows the result from biodiesel enforcement efforts. In year 2000 there was only 300 thousand barrel per day of biodiesel being produced and in just 10 years the value increased to 1.9 million barrel per day. The

consumption of biodiesel shows the similar pattern. That exponential growth resulted from a lot of enforcement worldwide especially from European countries.

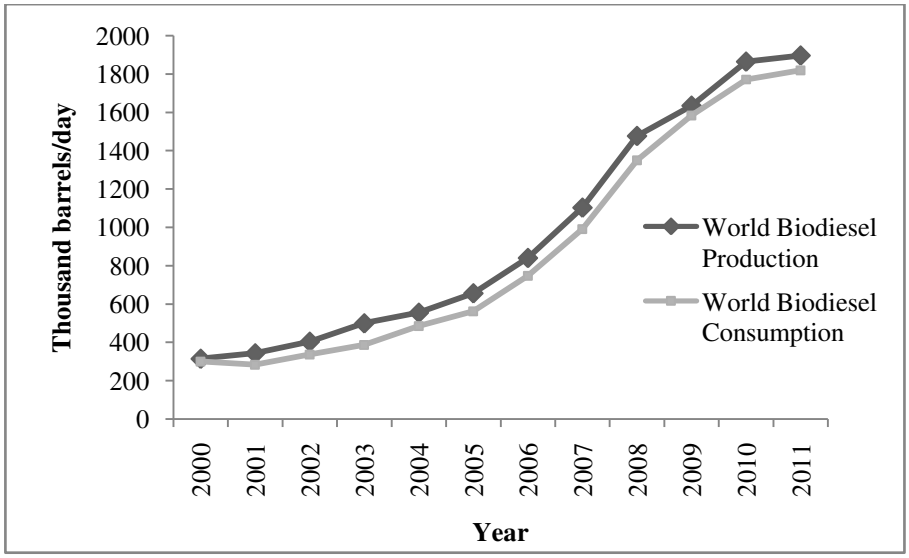

Figure 2.2: World Biodiesel Production and Consumption graph. (http://www.eia.gov)

European core biodiesel producers are Germany and France. The European countries supplied and consumed two over third of world biodiesel (ECOFYS report, 2011). Europe also plays an important role in biodiesel implementation as in April 2009; European Union endorsed a minimum binding target of 10% for biofuels use in transportation to be achieved by 2020, as a part of EU Directive 2009/28/EC on Renewable Energy. This Directive was a consequence on other Directives such as Directive 2003/96/EC on Energy Taxation and Directive 2003/30/EC. Direactive 2003/96/EC on Energy taxation allowed the tax exemption on promoting while Directive 2003/30/EC set 5.57% target of biofuels market penetration by 2010 (Sorda, Banse, & Kemfert, 2010).

2.2.2 Malaysia Biodiesel Trend

Malaysia has different scenario than Europe countries in biodiesel production and consumption. Despite the fact that Malaysia is one of the crude palm oil producers in the world, Malaysia tries to play a role in palm oil biodieselproduction. From European Biodiesel Board statistic data (www.ebb-eu.org), Malaysia produced 80 million tonnes of biodiesel in 2010 and exported 90 million tonnes of biodiesel to European Union and also United State of America countries. From this data it shows that Malaysia had successfully produced biodiesel for the export purpose but not for other local purpose like stated in Malaysia National Biofuel Policy. Malaysia launched National Biofuel Policy in 2006 that containts five strategic thrusts which are biofuel for transport, biofuel for industry, biofuel for technologies, biofuel for export and biofuel for cleaner environment. Followed after was a Malaysia Biofuel Industry Act 2007 that allowed the incentives for biodiesel production and consumption however, this act was not fully implemented and cause a lot of biodiesel industries when to bankruptcy as the authorities failed to monitor and support the industries. Beside, the palm oil biodiesel cannot compete with crude palm oil as Malaysia had a lot of attractive incentives on palm oil production and consumption, it suit to the name asone of the word palm oil producercountry (Biofuels in Malaysia, 2011).

2.3 BIODIESEL TRANSESTERIFICATION

Biodiesel production method was first patented in 1977 by Expedito Parente. Biodiesel is a clear yellowish liquid with almost similar viscosity as petroleum based diesel however biodiesel is non-flammable and non-explosive compared to diesel since it has high flash point than diesel. Biodiesel is produce from a reaction of triglycerides with alcohol. Figure 2.3 shows the biodiesel production chemical reaction. One mole triglyceride need three mole of alcohol to produce three mole fatty acid methyl ester (FAME) and one mole glycerol with the aid of catalyst.

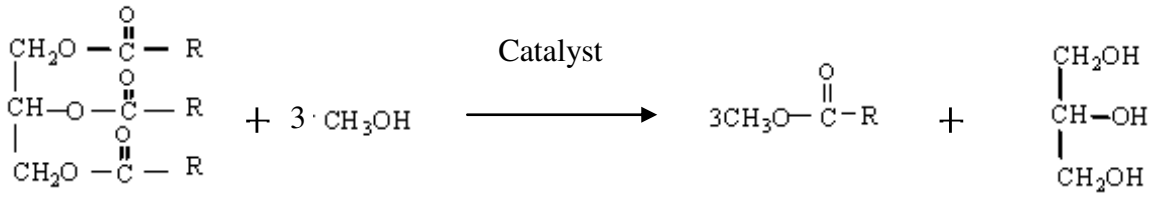

Figure 2.3: Biodiesel (FAME) chemical reaction mechanism

To date, there are a lot of technologies and researches on biodiesel. This includes any others study on catalyst, feedstock, reactor and reaction conditions.

2.3.1 Catalyst

Catalyst use to increase the reaction rate without being consume by the reaction. Catalyst can be divided to chemical catalyst and biocatalyst or also known as enzyme. Basically, chemical catalyst are acid and base catalsyts.

Chemical Catalyst

Chemical catalysts are the common catalysts used in biodiesel production. Industry sector favors chemical catalyst over biocatalyst because of it high yield and less production time. Chemical catalyst can be divided into acid and base catalysts. Leung & Guo (2006) studied on transesterification of biodiesel using sodium hydroxide (NaOH), potassium hydroxide (KOH) and sodium methoxide (CH_3ONa) found that among the three, CH_3ONa produced highest yield which is 89%. NaOH and KOH are conventional or primary alkali compared to CH_3ONa. Sodium methoxide was a secondary product of NaOH by deprotonation of methanol into NaOH.

At 70°C, 30 min reaction, methanol to oil molar ratio 7.5:1 and 600 rpm of mixing CH3ONa produced 3% higher biodiesel yield compared to NaOH and KOH using 1.3 wt %

of catalyst (Leung & Guo, 2006). The almost similar result was also reported by Rashid & Anwar (2008) that used KOH in transesterification of rapeseed oil. The optimum conditions that produced 96% of biodiesel was at 65°C, 6:1 methanol to oil molar ratio, 1 wt % of catalyst and 600 rpm of mixing in 70 minutes of reaction. Using NaOH alkali catalyst, Keera, Sabagh, & Taman (2011) managed to produce 98% of biodiesel in 1 hour of reaction with reaction conditions 60°C, 6:1 methanol to oil molar ratio, 1 wt % catalyst and 400 rpm mixing. Under high free fatty acid (FFA) and water, the alkali catalysts produces soap as the by product and make it difficult for separation process (Lam, Lee, & Mohamed, 2010).

Acid catalyst was one of the catalysts used in biodiesel production however, using acid catalyst, fatty acid was easily denatured due to high acidity conditions and need excess methanol to drive the transesterificationreaction for high yield production of biodiesel (Wu et al. 2008). Acid catalyst is less sensitive to FFA and water content that produced less glycerol but has slow reaction compared to alkali catalyst. Therefore, a combination of acid and alkali transesterification that was called acid-base catalysis was studied. Berchmans & Hirata (2008) reported that by using two-steps transesterification process, the high free fatty acid content (15%) in crude jatropha oil was reduced to less than 1% and produced 90% of methyl ester in 2 hours. 1 % w/w of Sulphuric acid (H_2SO_4) was used in first step for 1 hour at 50°C and at the second step 1.4% w/w of NaOH was used to produce biodiesel at 65°C.

Biocatalyst/Enzyme

Some drawbacks in the use of chemical catalyst in biodiesel production had drawn researcher's attention. Chemical catalysts producea large amount of by product which is glycerol. Glycerol is difficult to remove in separation process and generate wastewater problem (Lam et al., 2010). The use of enzyme in biodiesel production presents an alternative to the chemical catalyst. Enzyme has no or less by-product, easy glycerol separation, less sensitive to FFA content, work in mild reaction condition and recyclable (Kulkarni & Dalai, 2006). Enzyme also can overcome the high acid value level in biodiesel production (Li, Zong, & Wu, 2009). Lipase enzymes are the hydrolytic enzyme that

specialized in wide range reactions including transesterification reaction which is important to convert vegetable oil into fatty acid methyl ester in biodiesel making process (Noureddini et al., 2005). Wang et al. (2010) reported that Immobilized *R. Oryzae Lipase* successfully produced 90.5% biodiesel yield under optimum conditions which are methanol to oil molar ratio 4.8:1, 24 U/g enzyme activity, 37°C reaction temperature and 160 rpm for 12 hours. Using Immobilized Lipase from *Penicillium Expansum* Zong et al. (2009) produced 92.8% of methyl ester after 7 hours and the enzyme activity retained 68.4% of enzyme activity after 10 cycles. Immobilized enzyme has bright future in biodiesel production since the non-immobilized usually take 70 hours to reach reaction equilibrium.

Lipase enzyme can be produced from wide range of plants and organisms. Among the four types of lipase enzyme tested which are Lipase from *MucorMiehi*, Lipase from *Candida Antartica*, Lipase from *Pseudomonas Cepacia* and Lipase from *Candida Rugosa,* Pirajàn & Giraldo (2011) had reported that Lipase from *Candida Rugosa* produced highest yield of mono alkyl esters. 85% of methyl and ethyl esters were produced after 1 hour of reaction using 500 mg of Immobilized Lipase from *Candida Rugosa*with 50 g of palm oil, 14.5:1 ethanol to oil molar ratio and 1.0 g of water at 35°C by flow microcalorimetry.While Noureddini et al., (2005) investigated nine types of lipase enzymes including Lipase from *Candida Rugosa* using soybean oil and stated that the Lipase from *Pseudomonas Cepacia* resulted in highest yield of alkyl esters. 475 mg of Immobilized Lipase from *Pseudomonas Cepacia* mixed with 10 g of soybean oil, 17:5 methanol to oil molar ratio, 0.5 g of water at 35°C for 1 hour produced 67 mol % of alkyl esters. Both Pirajan & Giraldo (2011) and Noureddini et al. (2005) produced the almost similar optimum parameters and methyl ester yield. Compared to chemical catalyst, enzyme also can produce high quality of biodiesel in short time. However enzyme have some drawbacks which are expensive and has slow reaction than chemical catalyst. To overcome this drawback, enzyme immobilization was introduced.